

Supporting Information for:

**Multi-signal amplification electrochemical DNA biosensor  
based on exonuclease III and tetraferrocene**

Zhaojiang Yin <sup>#a</sup> , Hanfeng Cui <sup>#a</sup> , Qingxia Shu <sup>a</sup> , Chen Jin <sup>a</sup> , Yan Lin <sup>a</sup> , Jia Su <sup>b</sup> , HuiLian Huang <sup>a</sup> , Fusheng Liao <sup>a</sup> , Guangqiang Ma <sup>a</sup> , Nian Hong <sup>a</sup> , Yunfeng Jiang <sup>\*a</sup> , Hao Fan <sup>\*a</sup>

# Contributed to this work equally

\* Corresponding Author

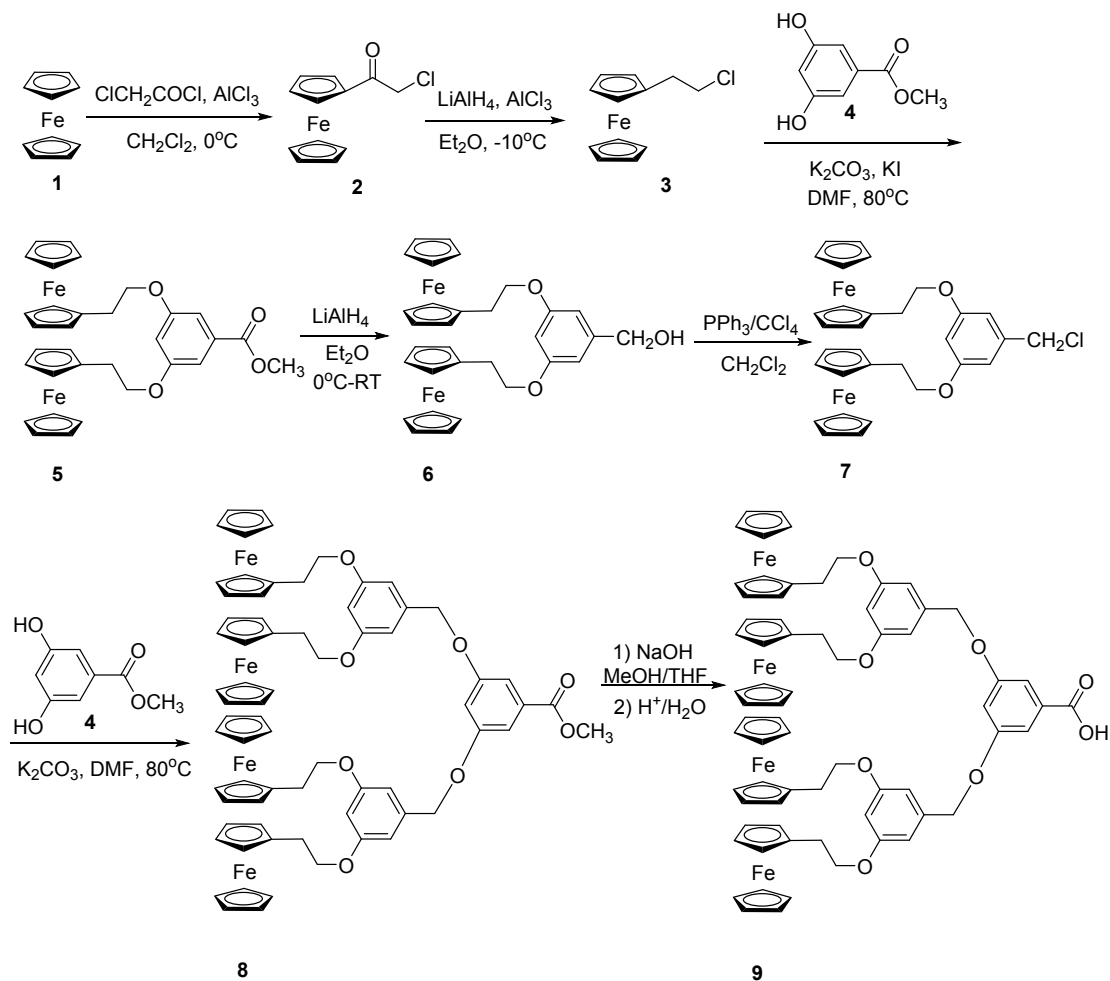
The Affiliated Hospital, Department of Pharmacy, JiangXi University of  
Traditional Chinese Medicine, JiangXi 330004, China<sup>a</sup>

South University of Science and Technology of China, Shenzhen 518055,  
China<sup>b</sup>

E-mail addresses: 254240787@qq.com (Yunfeng Jiang),

fanhao11@aliyun.com (Hao Fan)

**Scheme 1. The Synthetic of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic Acid (9).**



Procedure for the synthesis of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid **9**.

**$\alpha$ -chloroacetylferrocene 2.** To a solution of ferrocene (11.2 g, 60 mmol) in dichloromethane (60 mL) at 0°C, a solution of chloroacetyl chloride (3.8 mL, 50 mmol) and anhydrous aluminum trichloride (6.6 g, 50 mmol) in dichloromethane (80 mL) was added dropwise. After stirred for 8 h at room temperature, brine (100 mL) was added. The organic phase was separated and washed by brine. The resulting solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and concentrated in vacuum. The residue was purified by flash column chromatography to give orange crystal in 41.5% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600MHz):  $\delta$  4.88 (Cp-H, t,  $J = 2.0$  Hz, 2 H), 4.62 (Cp-H, t,  $J = 2.0$  Hz, 2 H), 4.46 ( $\text{CH}_2$ , s, 2 H), 4.25 (Cp-H, s, 5 H).

**2-Chloroethylferrocene 3.** A solution of  $\alpha$ -chloroacetylferrocene **2** (1.2 g, 4.58 mmol) in  $\text{Et}_2\text{O}$  (120 mL) was added dropwise to a solution of  $\text{LiAlH}_4$  (180 mg, 4.7 mmol) and  $\text{AlCl}_3$  (609 mg, 4.58 mmol) in  $\text{Et}_2\text{O}$  (90 mL) at -10 °C. After 1 h, the reaction was carefully quenched by addition of  $\text{H}_2\text{O}$  (20 mL) and diluted with brine (100 mL). The aqueous layer was extracted with ethyl acetate (3×50 mL). The combined organic layer was washed with brine (3×50 mL), dried over anhydrous sodium sulfate, and

concentrated. The residue was purified by a silica gel column with ethyl acetate-Petroleum ether (1:15) as the eluent to afford yellow product **3** (0.76 g, 67%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) δ 4.16 (Cp-H, s, 7H), 4.13 (Cp-H, s, 2H), 3.62 (CH<sub>2</sub>-O, t, *J* = 7.2 Hz, 2H), 2.84 (Fc-CH<sub>2</sub>-, t, *J* = 7.2 Hz, 2H).

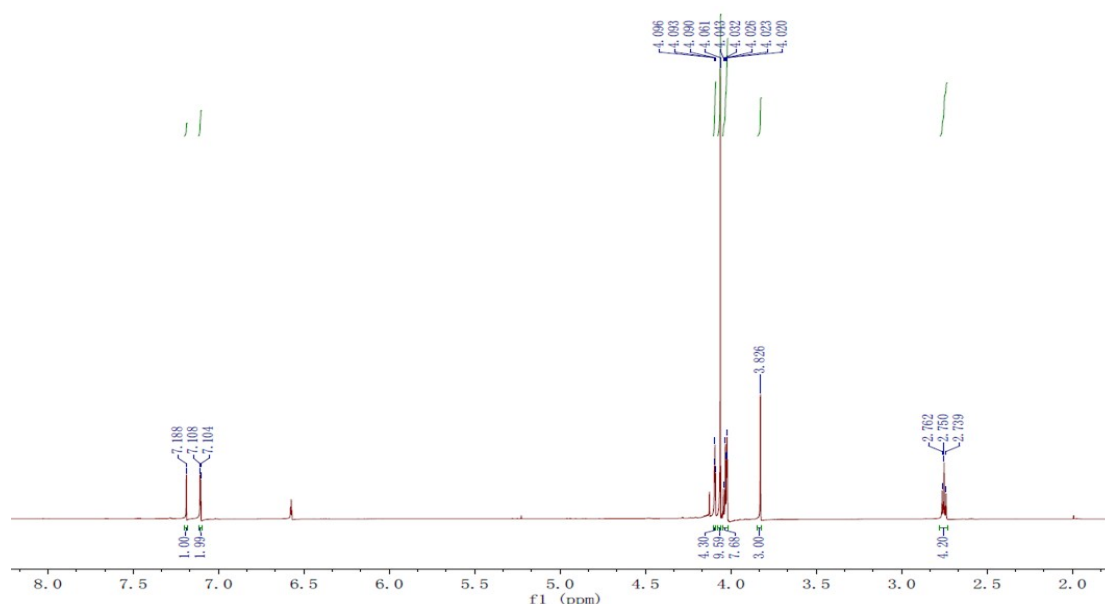
Methyl 3, 5-bisferrocenethoxybenzoate **5**. 2-Chloroethylferrocene **3** (548.1mg, 2.21 mmol), methyl 3,5-dihydroxybenzoate **4** (154.6 mg, 0.92 mmol), anhydrous potassium carbonate (552 mg, 4 mmol) and potassium iodide (10 mg, 0.06 mmol) in DMF (4 mL) was mixed together and stirred at 80 °C until disappearance of starting material was observed by TLC. The mixture was diluted with ethyl acetate (80 mL) and washed with water (3×30 mL) and brine (3×30 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave **5** as yellow solid (398 mg, 73%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) δ 7.19 (Ar-H, s, 1H), 7.11 (Ar-H, d, *J* = 2.4 Hz, 2H), 4.09 (Cp-H, t, *J* = 1.5 Hz, 4H), 4.06 (Cp-H, s, 10H), 4.04-4.02 (Cp-H, -CH<sub>2</sub>-O, m, 8H), 3.82 (O-CH<sub>3</sub>, s, 3H), 2.75 (Fc-CH<sub>2</sub>-, t, *J* = 7.2 Hz, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz) δ 166.9, 159.9, 131.9, 107.7, 106.8, 84.6, 68.8, 68.6, 68.6, 67.5, 52.3, 29.5. HRMS (ESI) Calcd. for C<sub>32</sub>H<sub>32</sub>Fe<sub>2</sub>O<sub>4</sub> [M]<sup>+</sup>: 592.0999; found 592.0991.

(3, 5-bisferrocenethoxyphenyl)methanol **6**. To a solution of LiAlH<sub>4</sub> (28.8 mg, 0.76 mmol) in anhydrous ether (10 mL), a solution of methyl 3, 5-bisferrocenethoxybenzoate **5** (50 mg, 0.084 mmol) in anhydrous ether (15 mL) was added dropwise at 0 °C. The mixture was stirred at room temperature until the disappearance of the starting material. And then the reaction was carefully quenched by H<sub>2</sub>O (20 mL), the aqueous layer was separated and extracted with diethyl ether (3×25 mL). The combined organic layer was washed with brine (3×50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography to give orange crystal **6**. Yields: 89.0%. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) δ 6.55 (Ar-H, s, 2H), 6.43 (Ar-H, s, 1H), 4.64 (ArCH<sub>2</sub>-O, s, 2H), 4.20-4.13 (Cp-H, m, 18H), 4.10 (-CH<sub>2</sub>-O, t, *J* = 7.2 Hz, 4H), 2.84 (Fc-CH<sub>2</sub>-, t, *J* = 7.2 Hz, 4H). HRMS (ESI) Calcd. for C<sub>31</sub>H<sub>32</sub>Fe<sub>2</sub>O<sub>3</sub> [M]<sup>+</sup>: 564.1050; found 564.1037.

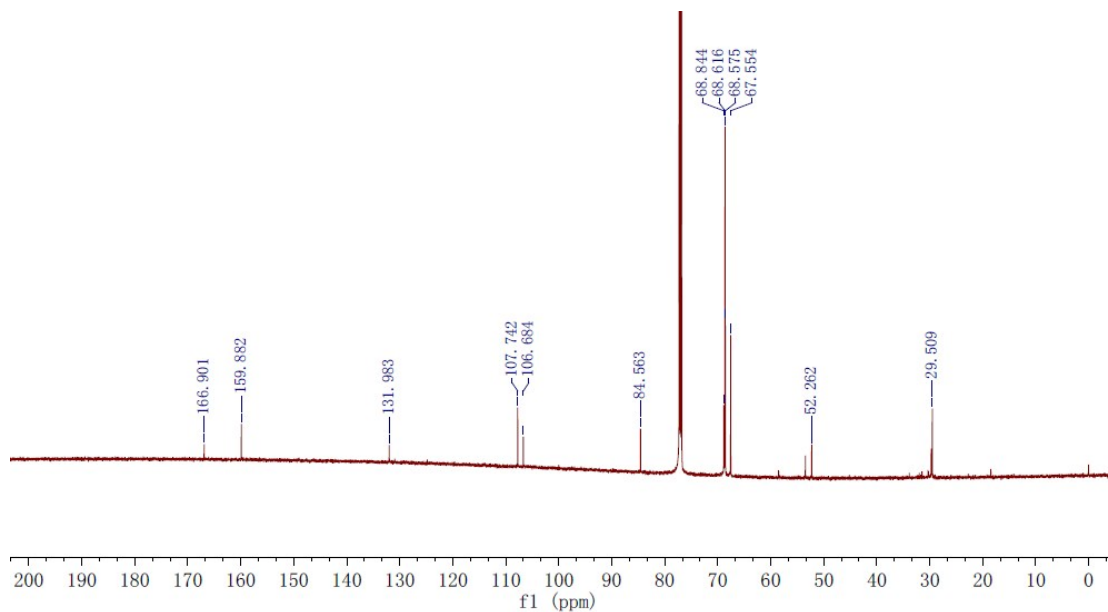
3, 5-bisferrocenethoxybenzyl chloride **7**. To a solution of PPh<sub>3</sub> (0.4313 g, 1.65 mmol) in CCl<sub>4</sub> (10 mL), a solution of (3, 5-bisferrocenethoxyphenyl)methanol **6** (0.7739 g, 1.37 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10mL) was added dropwise. The mixture solution was refluxed for 30 min and observed by TLC. The mixture was diluted with dichloromethane (40 mL) and washed with water (3×20 mL) and brine (3×20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave **7** as dark orange solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) δ 6.55 (Ar-H, d, *J* = 1.8 Hz, 2H), 6.43 (Ar-H, t, *J* = 2.4 Hz, 1H), 4.53 (ArCH<sub>2</sub>-O, s, 2H), 4.18 (Cp-H, t, *J* = 1.8 Hz, 4H), 4.15(Cp-H, s, 10H), 4.11 (Cp-H, t, *J* = 1.8 Hz, 4H), 4.09 (-CH<sub>2</sub>-O, t, *J* = 7.2 Hz, 4H), 2.83 (Fc-CH<sub>2</sub>-, t, *J* = 7.2 Hz, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz) δ 160.3, 139.5, 107.1, 101.3, 84.6, 68.7, 68.6, 68.6, 67.5, 46.2, 29.2. HRMS (ESI) Calcd. for C<sub>31</sub>H<sub>31</sub>ClFe<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 582.0711; found 582.0731.

Methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate **8**. To a solution of methyl 3,5-dihydroxybenzoate **4** (6.6 mg, 0.039 mmol) and anhydrous  $K_2CO_3$  (21.5 mg, 0.156 mmol) in 10mL of DMF, 3, 5-bisferrocenethoxybenzyl chloride **7** (50 mg, 0.085mmol) was added. The reaction was stirred at 80 °C under nitrogen atmosphere and observed by TLC. The mixture was diluted with ethyl acetate (30 mL) and washed with brine (3×15 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave **8** as dark yellow solid.  $^1H$  NMR ( $CDCl_3$ , 600 MHz)  $\delta$  7.30 (Ar-H, d,  $J = 2.4$  Hz, 2H), 6.82 (Ar-H, t,  $J = 2.4$  Hz, 1H), 6.59 (Ar-H, d,  $J = 2.4$  Hz, 4H), 6.45 (Ar-H, t,  $J = 2.4$  Hz, 2H), 5.01 (ArCH<sub>2</sub>-O, s, 4H), 4.18 (Cp-H, t,  $J = 1.2$  Hz, 8H), 4.15-4.09 (Cp-H and -CH<sub>2</sub>-O, m, 36H), 3.91 (-COOCH<sub>3</sub>, s, 3H), 2.83 (Fc-CH<sub>2</sub>-, t,  $J = 7.2$  Hz, 8H).

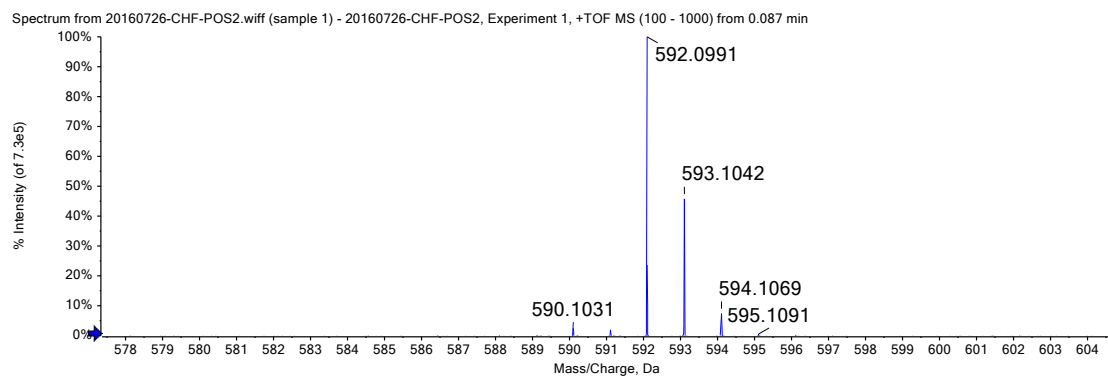
3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid **9**. To a solution of methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate **8** (50 mg, 0.04 mmol) in THF/MeOH (10mL, v/v = 1: 1) was added 10% aqueous sodium hydroxide (2 mL) at room temperature. The mixture was kept stirring overnight, the pH was adjusted to about 5 with dilute hydrochloric acid. The resulting orange crystals were collected by filtration, washed with water, and dried under vacuum. A pure sample was obtained by recrystallization from dichloromethane/ petroleum ether to give **9** (32 mg, 65% yield) as dark yellow solid.  $^1H$  NMR ( $CDCl_3$ , 600 MHz)  $\delta$  7.32 (Ar-H, s, 2H), 6.85 (Ar-H, s, 1H), 6.59 (Ar-H, s, 4H), 6.44 (Ar-H, s, 2H), 5.01 (ArCH<sub>2</sub>-O, s, 4H), 4.20-4.11 (Cp-H, m, 36H), 4.09 (-CH<sub>2</sub>-O, t,  $J = 7.2$  Hz, 8H), 2.83 (Fc-CH<sub>2</sub>-, t,  $J = 6.6$  Hz, 8H). HRMS (ESI) Calcd. for  $C_{69}H_{66}Fe_4O_8$   $[M]^+$ : 1246.2155; found 1246.2166.



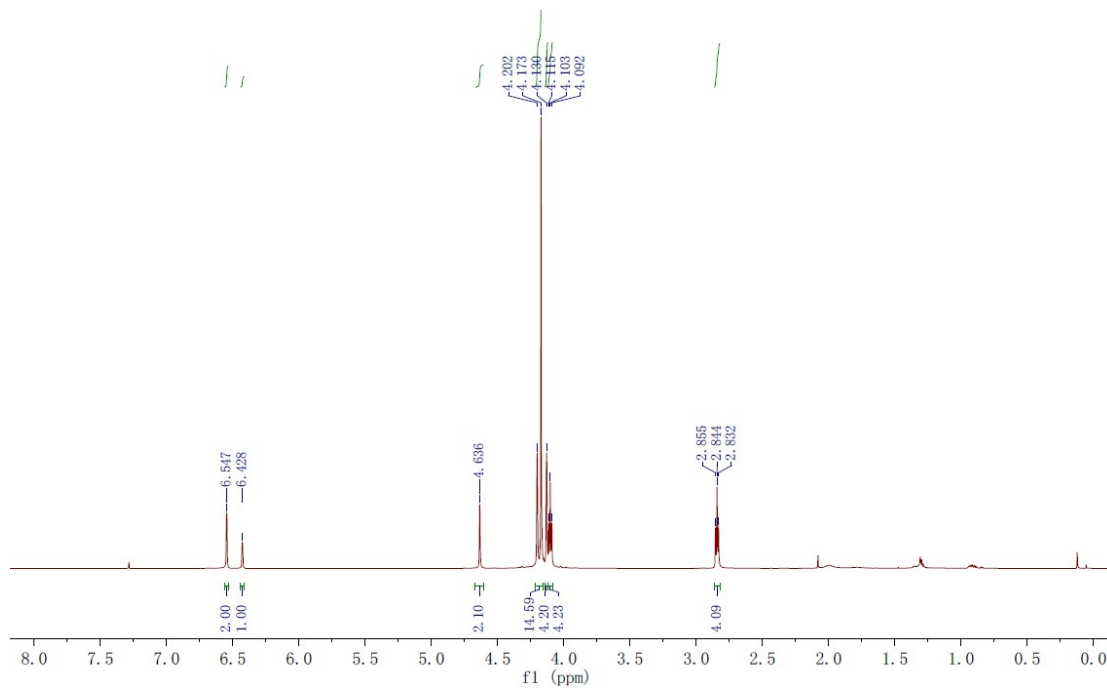
$^1H$  NMR of methyl 3,5-bisferrocenethoxybenzoate **5**



$^{13}\text{C}$  NMR of methyl 3,5-bisferrocenethoxybenzoate **5**

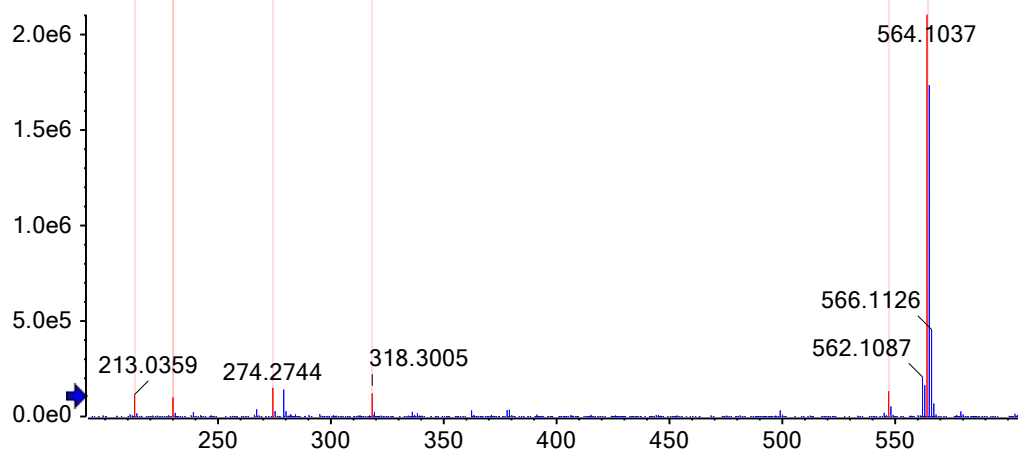


HRMS of methyl 3,5-bisferrocenethoxybenzoate **5**

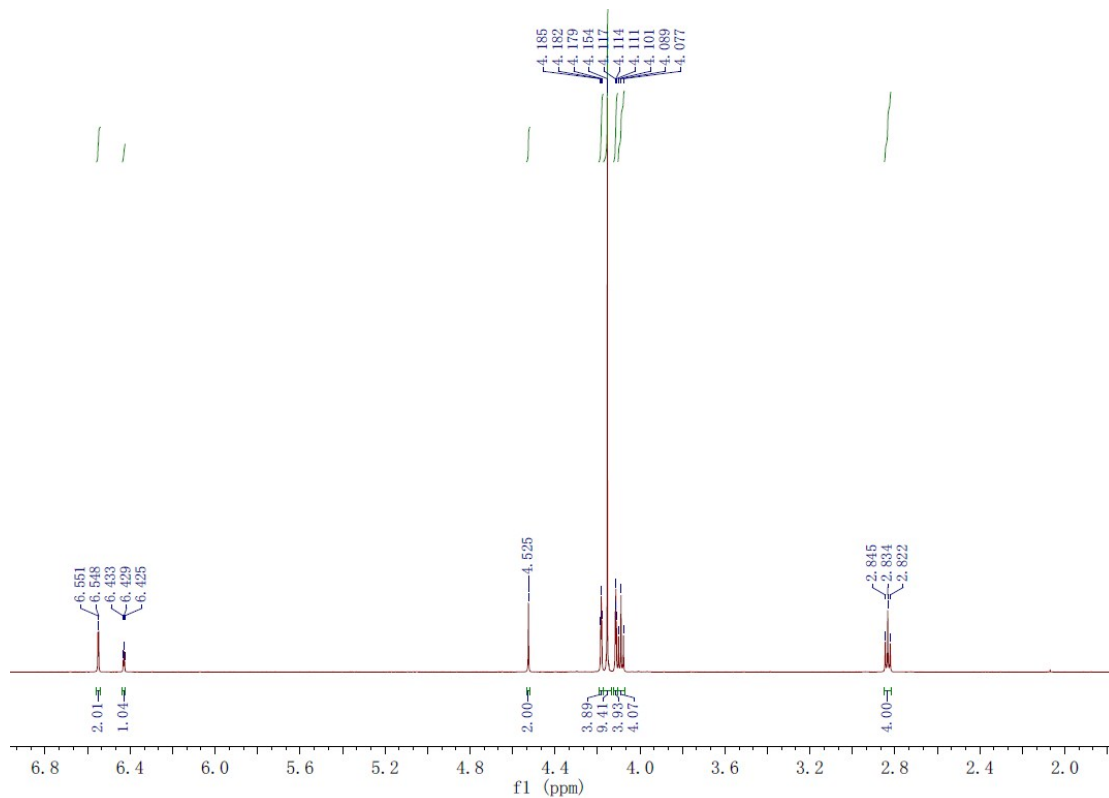


<sup>1</sup>H NMR of (3, 5-bisferrocenethoxyphenyl)methanol **6**

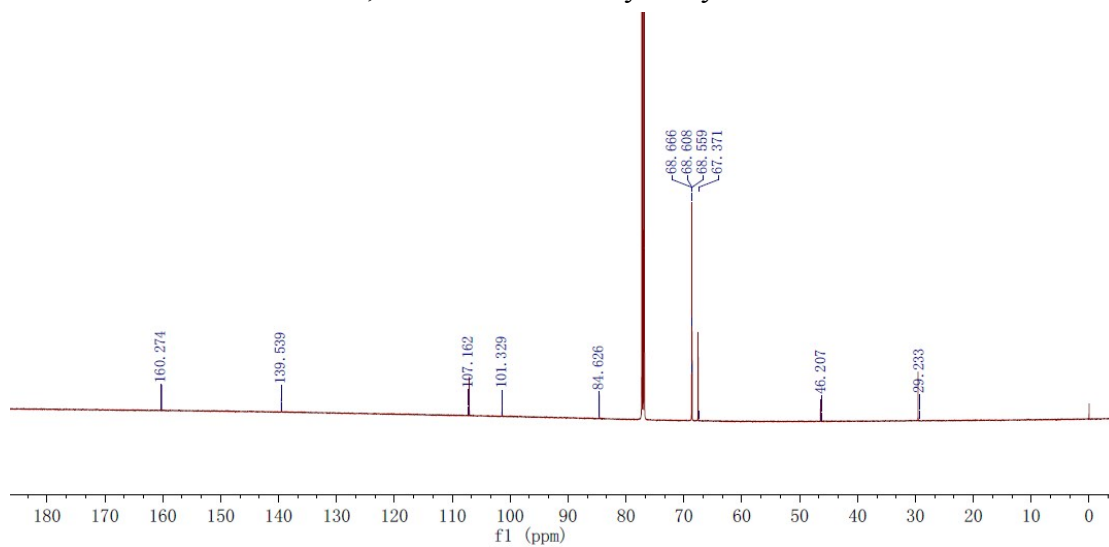
Spectrum from 20161215-POS-H161209-1.wiff (samp...m 0.104 min, noise filtered, Gaussian smoothed)



HRMS of (3, 5-bisferrocenethoxyphenyl)methanol **6**

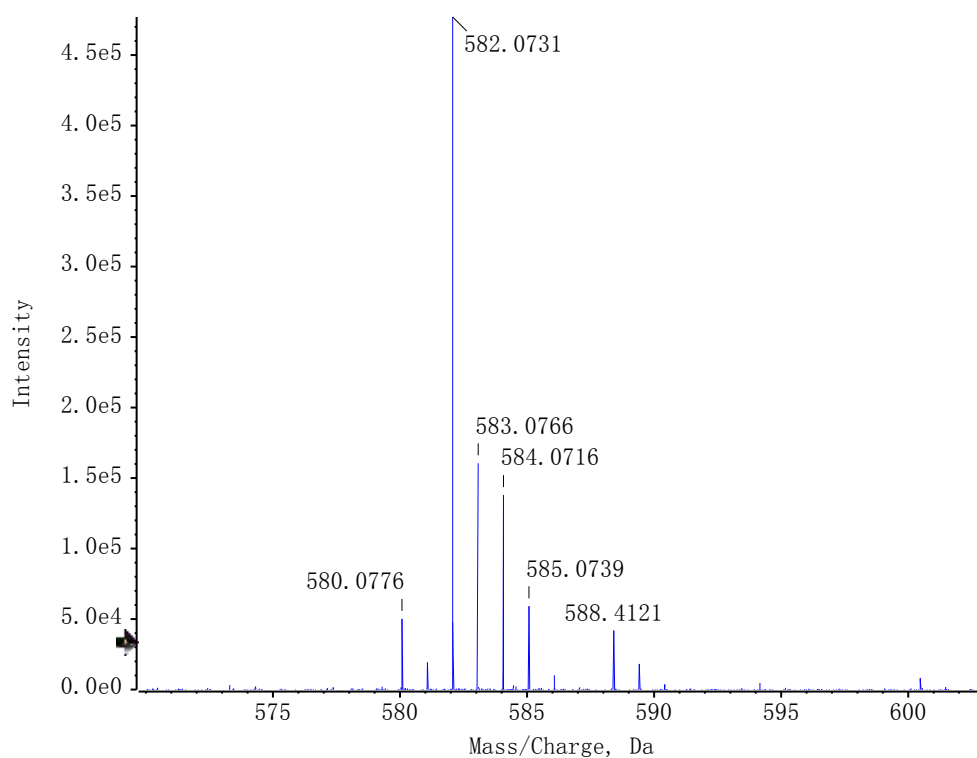


$^1\text{H}$  NMR of 3, 5-bisferrocenethoxybenzyl chloride **7**

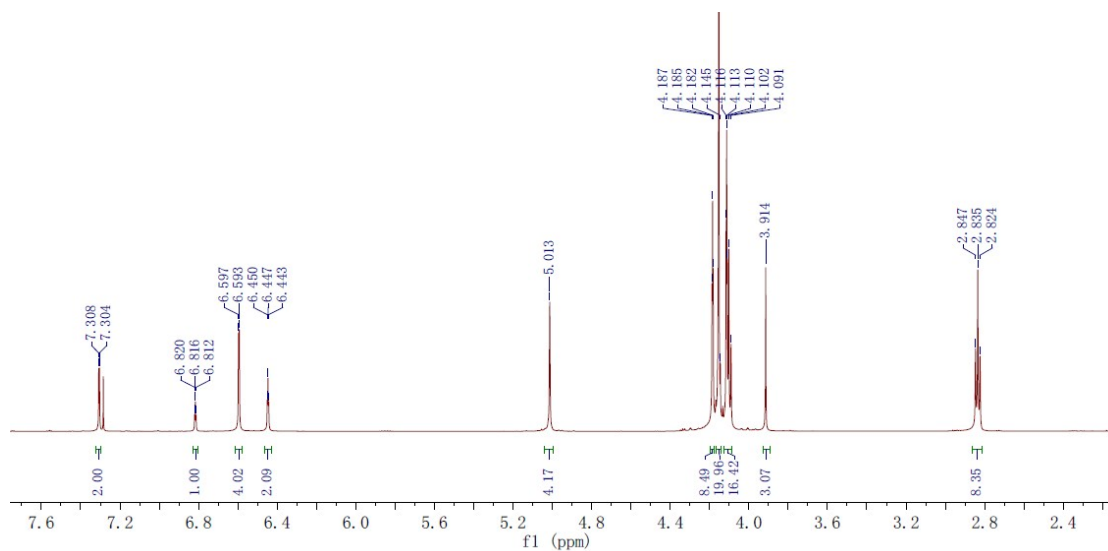


$^{13}\text{C}$  NMR of 3, 5-bisferrocenethoxybenzyl chloride **7**

Spectrum from 20170418-POS-H170414.wif..., +TOF MS (100 - 3000) from 0.101 min

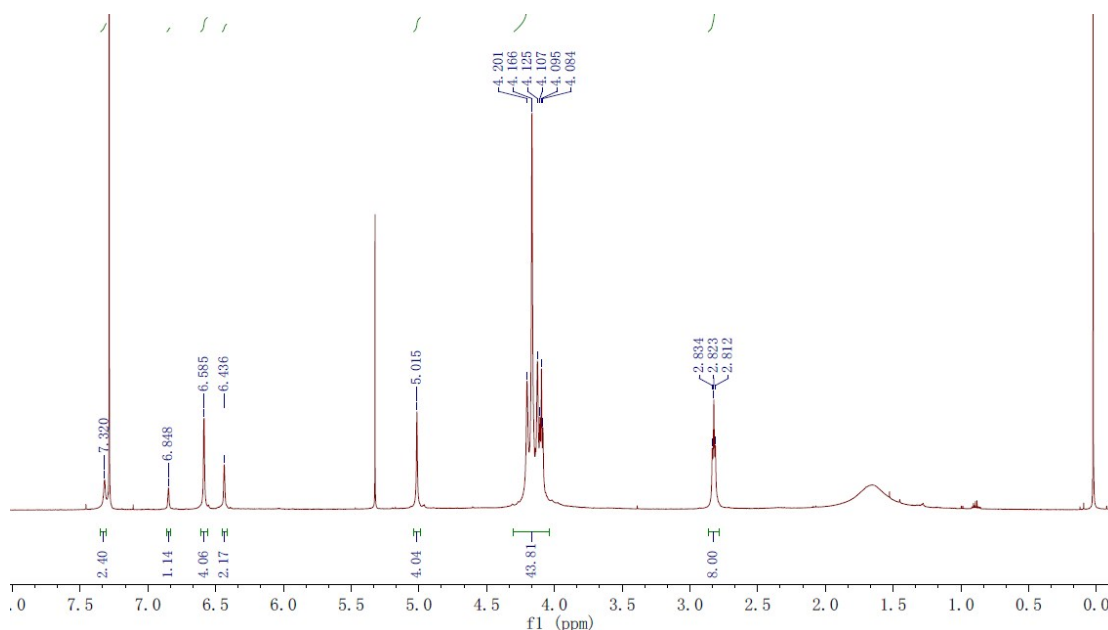


HRMS of 3, 5-bisferrocenethoxybenzyl chloride **7**



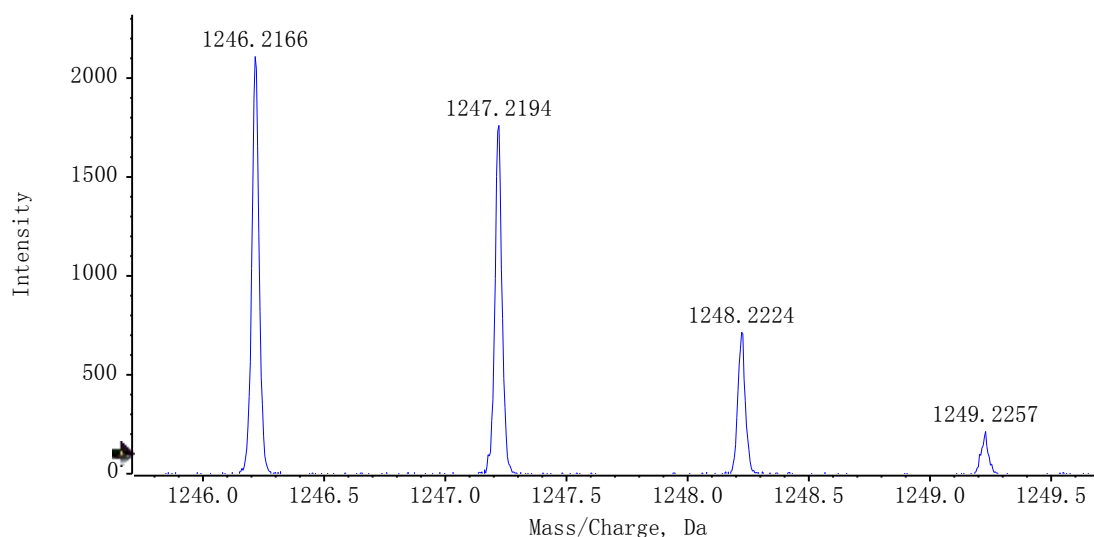
<sup>1</sup>H NMR of methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate **8**





### <sup>1</sup>H NMR of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid **9**

Spectrum from 20170620-POS-H170619.wiff (sam...TOF MS (100 - 1500) from 0.326 to 0.358 min)



### HRMS of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid **9**

## 2. Real sample analysis

We selected the detection of PCR amplicons from the *Cordyceps sinensis* gene to test the real applicability of our DNA sensor. By using PCR protocol, we obtained DNA targets of 158 bp that could be directly detected by the sensor. We found that this sensor could selectively identify as few as  $1 \times 10^{-13}$  LYC DNA in PCR environment, which suggested that the DNA sensing was a promising method for the rapid detection of PCR product. We used NanoDrop 2000/2000C to determine the concentration of PCR product.

The PCR program was set up as 94°C 3 minutes; 90°C 45 seconds, 65°C 45 seconds,

72°C 1 minute, 35cycles; 72°C 5 min.

Target (PCR product):

5'-

TATTTGCTTGCTTCTTGACTGAGAGATGCCACTGCGACAGGAGGGTCTGG  
GGGTCCTCCGGCGGGCGCCCTGGTCCGGGCCCTGGGGCGCCGGGGCGGTC  
CCGCCGAGGCAACTGCTGTGGTGTTCGCAGGGGGTTTGGGAGTGGTGACT  
CGATAATA-3'

A forward—Hsprp1 primer(5'-ATTATCGAGTCACCACTCCCAAACCCCC-3')

A reverse—Hsprp2 primer (5'-ATTTGCTTGCTTCTTGACTGAGAGATGCC-3')

The probe:

5'- Tetraferrocene-TTTTAACTAAAGCAGTTGCCTCGGCGGGACCGCCC -3'